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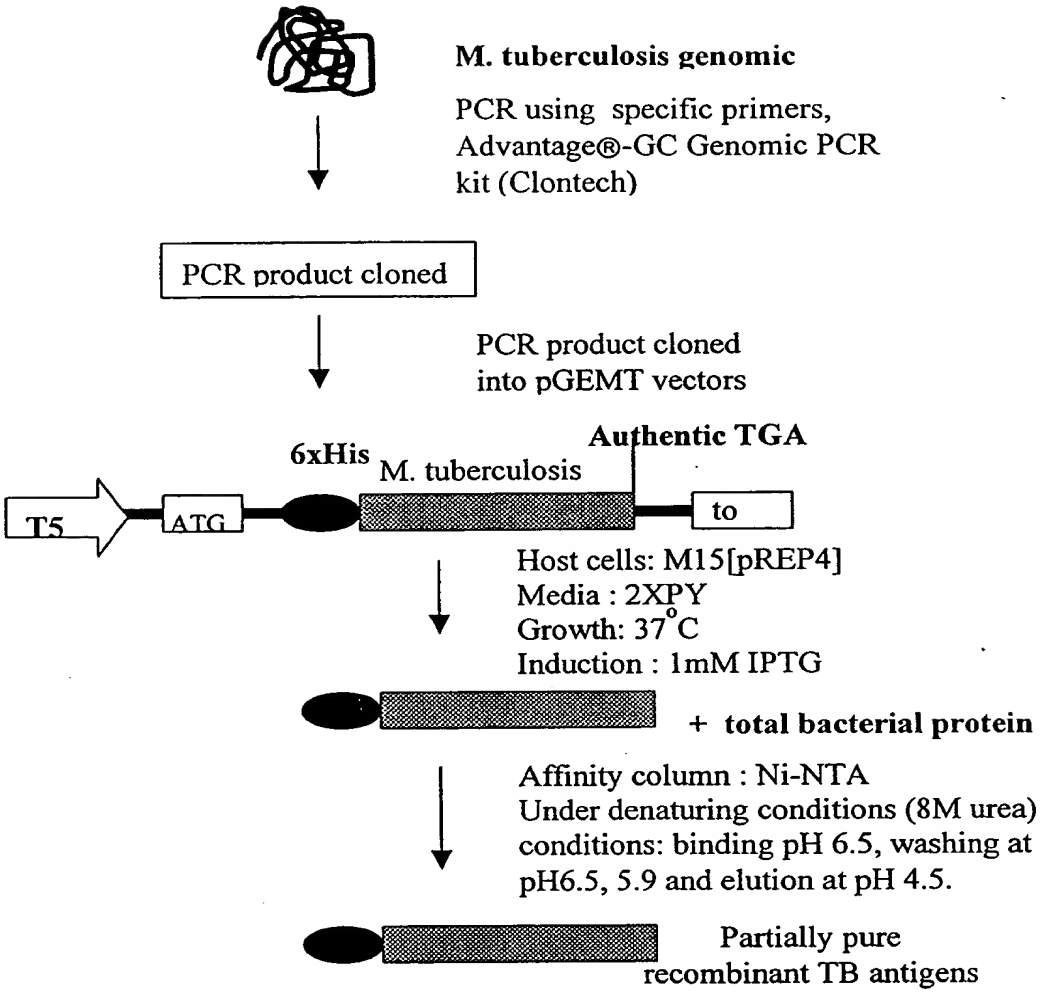
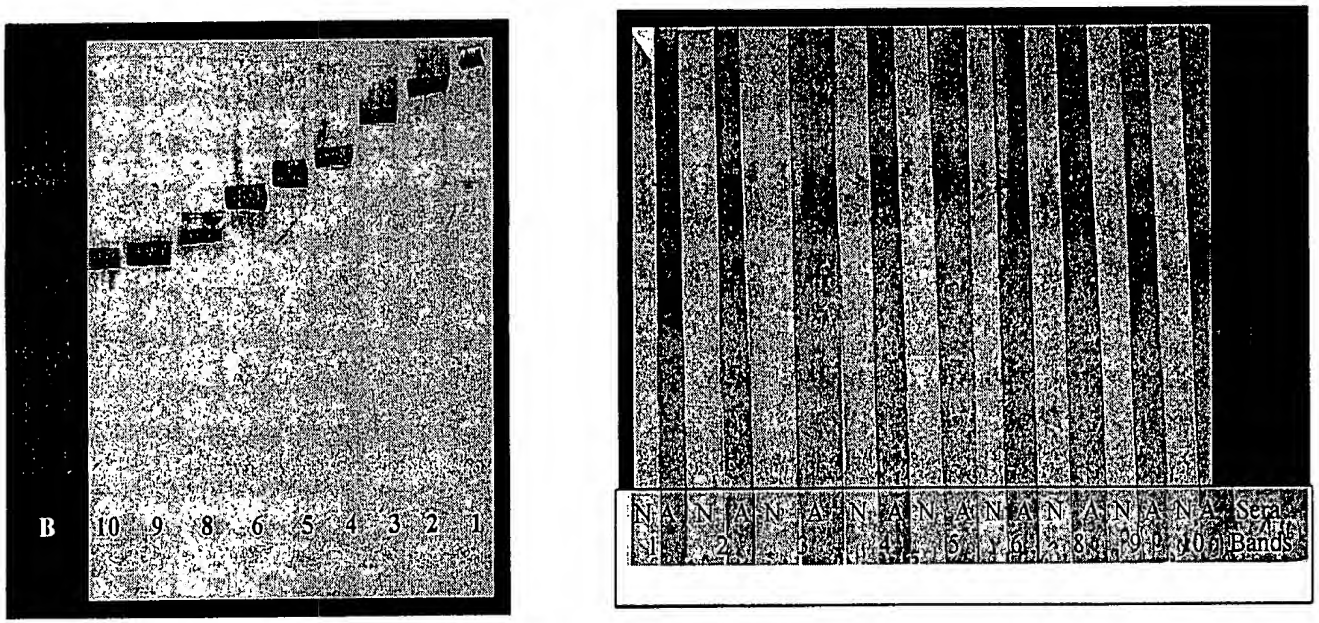



Fig.1 Strategy for the isolation and expression of M.tuberculosis protein antigens.




(A) (B)

Fig.2. (A) Gel purified and concentrated M. tuberculosis protein bands (B.1, 2, 3, 4, 5, 6, 8, 9, 10) blotted onto PVDF membrane were excised for N-terminal sequencing. (B) Concentrated M. tuberculosis protein bands blotted onto nitrocellulose membrane and immuno-screened using pooled normal (N) and active (A) sera respectively. Positive bands (arrows) were observed with A but not with N.

**Fig. 3** Result of homology search against the GenBank protein sequence databases. Proteins showing the highest homology to the M. tuberculosis protein bands are as shown.

| Relative molecular weight (kDa) | Sequence from N-terminal sequencing | Match (GenBank) |
|---|---|---|
| B.4 | SKLIEYDELALEAME | db: $_{2}$SKLIEYDETARHAME$_{16}$ 55.74kDa, groEL1/protein cpn60 [16], pID=g44601, X60350 (80% match) |
| B.5 | AKTIAYDEEARV | db: $_{2}$AKTIAYDEEA$_{10}$ 56.728 kDa, CHAPERONIN2, groEL2, GenBank pID=g15000, MTTCWPA_3 (100% match) |
| B.6 | AEVDAYKFDPDAVD | db: $_{161}$AEFDAYRRDPMA$_{172}$ Probable exported protease, has signal sequence, very similar to three proteases / peptidases from Streptomyces, pID=e235164, MTCY427.04c (51% match) |
| B.9 | AEYTLPDLDWDYG | db: $_{2}$AEYTLPDLDWDYG$_{14}$ 23.0 kDa, superoxide dismutase, pID=g581379, MTSOD4 (100% match) |
| B.10 | MEIDILAVAAP | db: $_{117}$IEVDLLDLDAP$_{127}$ 33 kDa, mycocerosic acid synthase [17], pID=g149978, M95808 (56.9% match) |
| MMP | ATTLPVQRHDARL | db:ATLPVQRHPRSL 14/16 kDa [18], pID=g244562, M76712 |

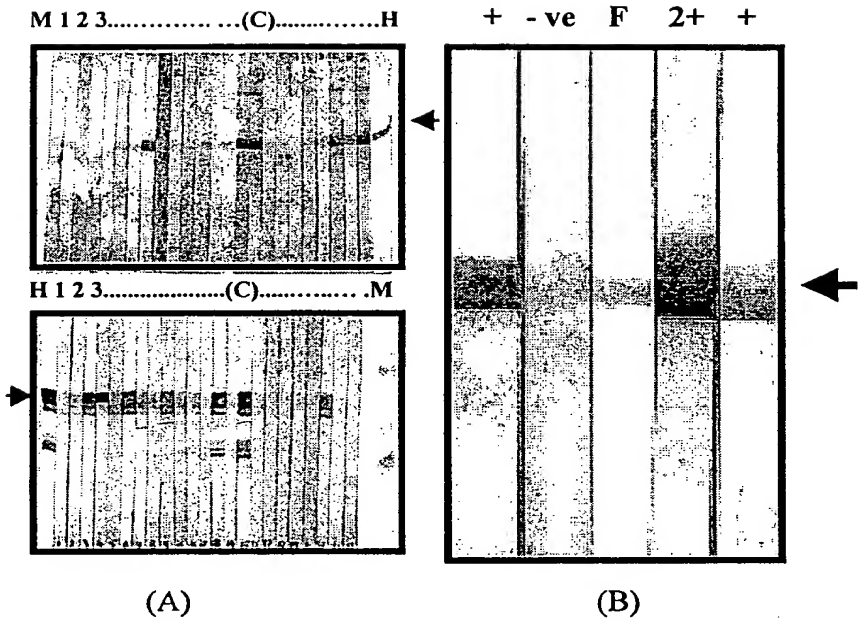



Fig. 4. Western screening of recombinant M. tuberculosis antigens. (A) Arrows indicate the position of the recombinant antigens on the membrane. M= Kaleidoscope protein Marker and H= strip probed with anti-RGSHis, C= a positive control of strips probed with known human serum reactive to the specific recombinant antigen. (B) Reactivity is estimated based on the intensity of band on

**Fig 5. Percentage of reactivity of recombinant TB antigens against different sera panels.** A known 38kDa antigen [20, 21] of M. tuberculosis was included in the screening. The gene (GeneBank Accession # M30046) for this antigen was cloned, expressed in pQE30 and partially purified as described in section E. Also shown are the percentage of reactivity of sera samples detected by a commercially available rapid TB diagnostic kit from ICT (Amrad).

| Sera Panel: Recombinant antigens: | Uninfected (normal) | Active TB (Extra-Pulmonary) | Active TB (Pulmonary) | Inactive |
|---|---|---|---|---|
| B.4 | 5% | 55% | 47.8% | 22.7% |
| B.5 | 25% | 35% | 39.1% | 27.3% |
| B.6 | 0% | 5% | 52.2% | 9.1% |
| B.9 | 0% | 25% | 17.4% | 18.2% |
| B.10 | 0% | 5% | 26.1% | 0% |
| MMP | 0% | 25% | 8.7% | 4.5% |
| C17 | 0% | 15% | 13.0% | 4.5% |
| 38 kDa | 0% | 40% | 39.1% | 18.2% |
| ICT TB Kit | 0% | 55% | 52.2% | 13.6% |

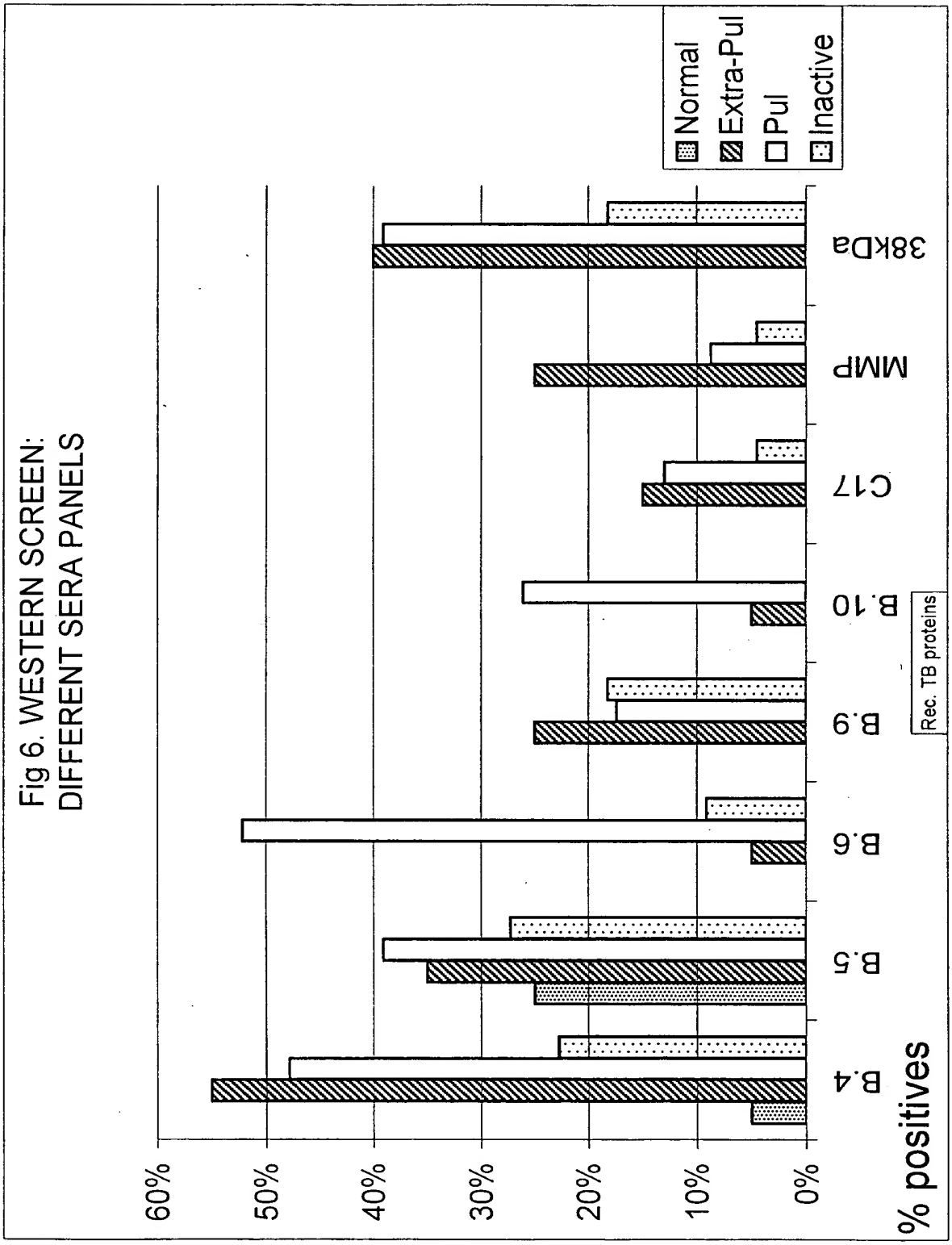



Fig 6. WESTERN SCREEN:
DIFFERENT SERA PANELS

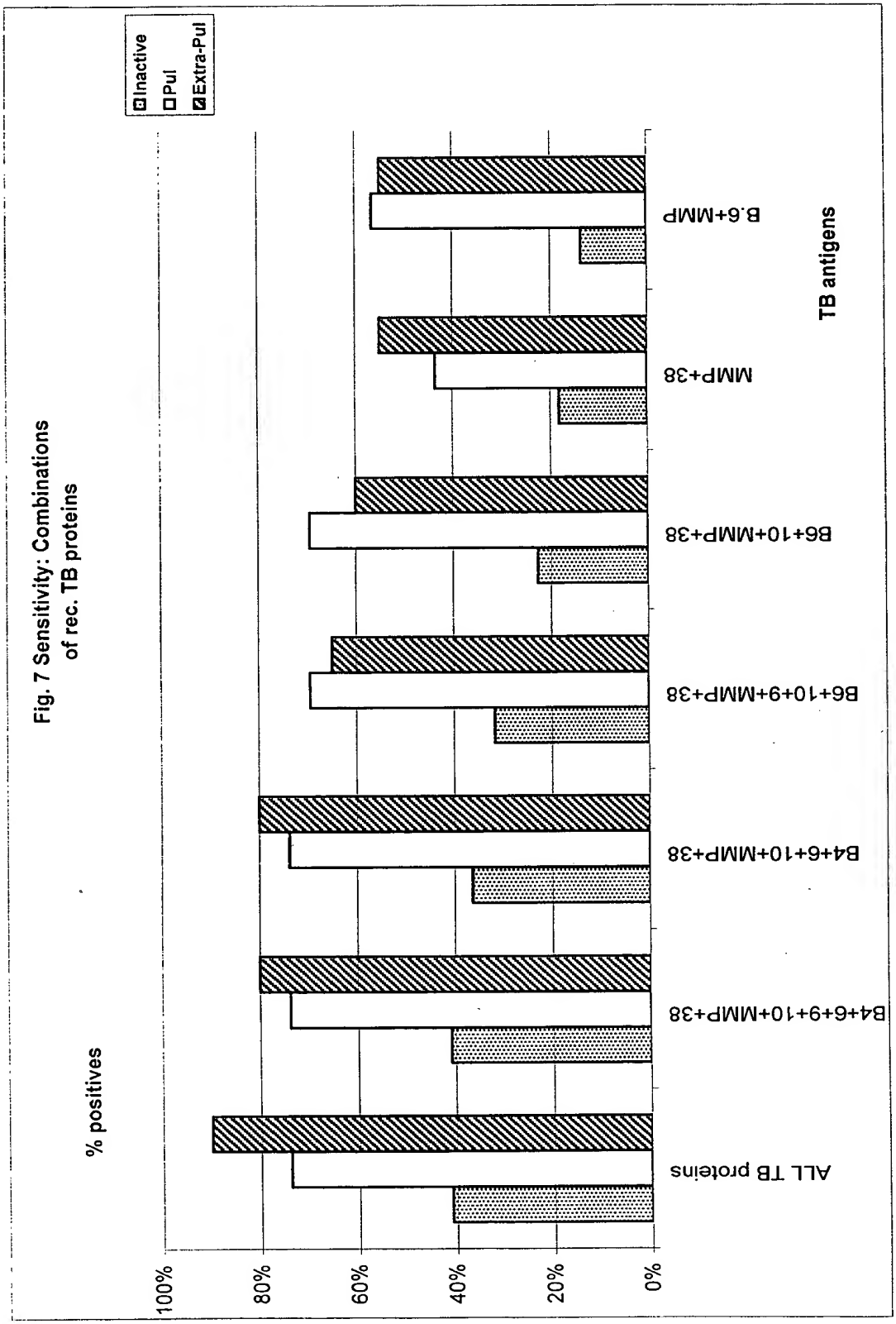



Fig. 7 Sensitivity: Combinations of rec. TB proteins

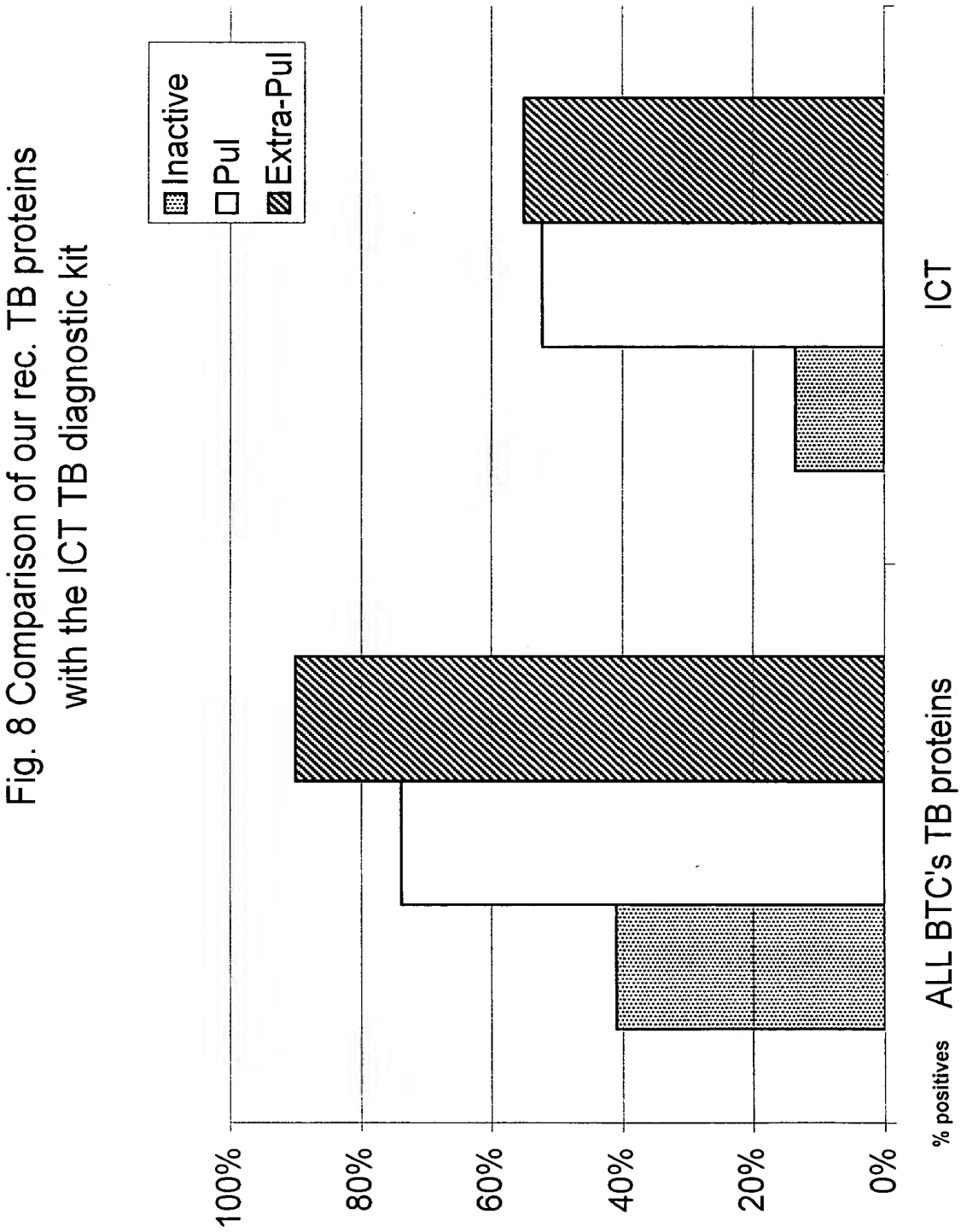



Fig. 8 Comparison of our rec. TB proteins with the ICT TB diagnostic kit

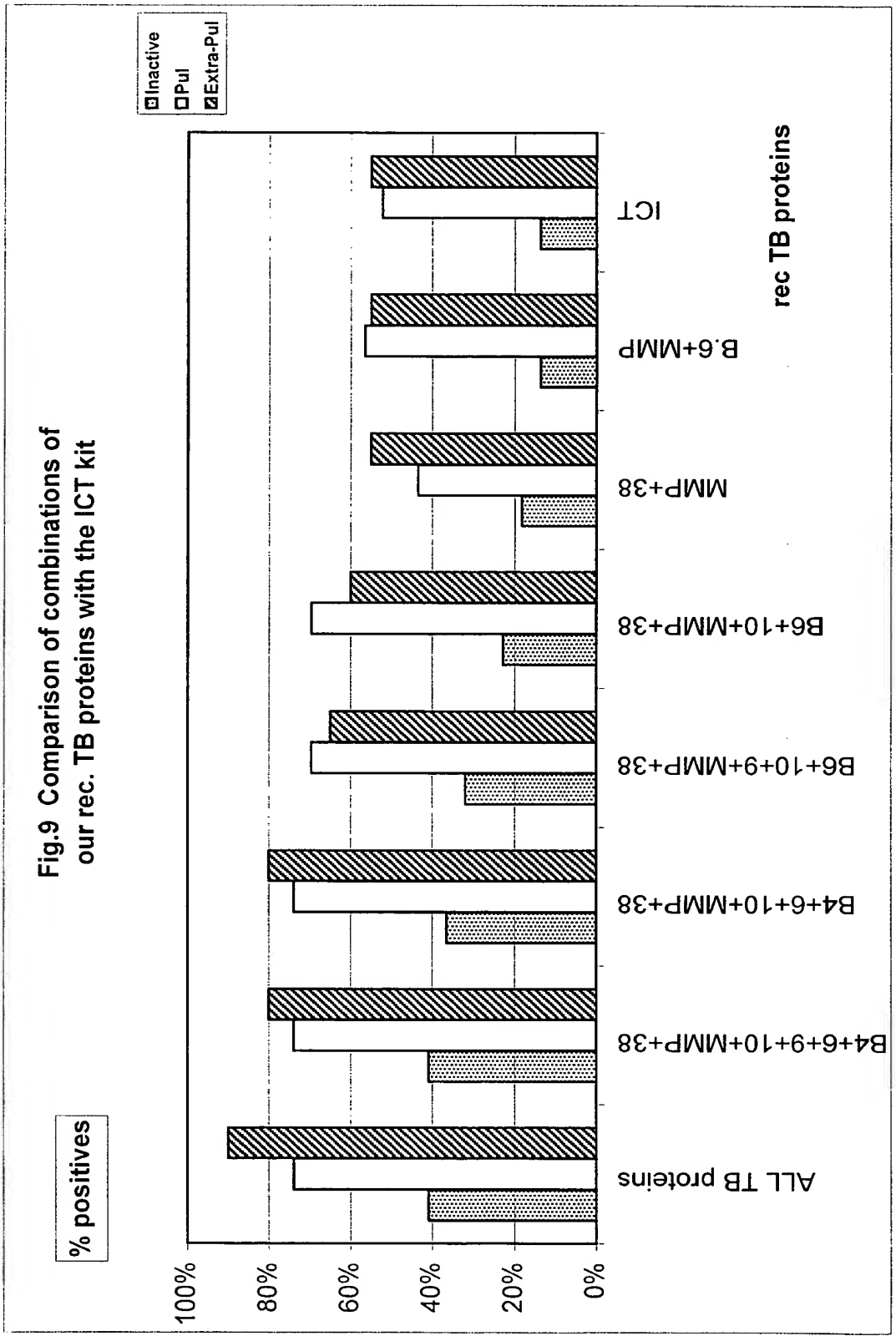



Fig.9 Comparison of combinations of our rec. TB proteins with the ICT kit